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(54) **Title:** METHOD TO POTENTIATE THE THERAPEUTIC EFFICACY OF TAXANE AND DERIVATIVES THEREOF

(57) **Abstract:** The use of estramustine phosphate and its metabolites estramustine and estromustine allow to potentiate the therapeutic efficacy of taxanes by both improving their pharmacokinetic and pharmacodynamic profile through the inhibition of (CYP)2C8 and (CYP)3A4 enzymes, both responsible for the metabolism of the taxanes; formulations of estramustine phosphate and metabolites, combinations of these latter with taxanes and therapeutic methods of treatment comprising them as a combined therapy are also disclosed.

METHOD TO POTENTIATE THE THERAPEUTIC EFFICACY OF TAXANE AND DERIVATIVES THEREOF

The present invention relates to a method for potentiating the therapeutic efficacy of taxanes and, more particularly, to a method for potentiating the therapeutic efficacy of taxanes by improving the pharmacokinetic as well as pharmacodynamic profile of the taxanes themselves.

Among the family of taxanes, widely known as antitumor agents, is taxol (paclitaxel), a natural product derived from the yew tree having a significant clinical activity against a broad range of tumor types such as, for instance, breast, lung, head and neck, bladder and platinum-refractory ovarian carcinoma (Rowinsky, 1997).
When administered to patients, the drug is readily metabolized to several hydroxylated products. All metabolites so far characterized have been found to be less cytotoxic than paclitaxel itself (Harris, 1994; Kumar, 1995).
Substantially analogous metabolic pathway, hence leading to metabolites which retain cytotoxicity, but to a lesser extent, has also been observed for other taxanes such as, for instance, taxotere (docetaxel).

Previous studies in vitro identified that taxanes are extensively metabolized, through hepatic route, by the action of cytochrome P450 enzymes.
Within this family (CYP)2C8 and (CYP)3A4 are the main enzymes which appear to be involved in taxanes metabolism (Cresteil, 1994; Rahman, 1994).
When referring to paclitaxel, in particular, the formation of the metabolite 6α-hydroxypaclitaxel is catalyzed by (CYP)2C8 whereas the other metabolite p-hydroxyphenyl-C3'-paclitaxel is formed by (CYP)3A4.
6α-Hydroxypaclitaxel or, alternatively, p-hydroxyphenyl-C3'-paclitaxel have been observed to be the pre-dominant metabolites (Desai, 1998).
Dihydroxypaclitaxel is then formed by subsequent hydroxylation of p-hydroxyphenyl-C3'-paclitaxel by CYP2C8 and of 6α-hydroxypaclitaxel by CYP3A4. The roles of both enzymes are summarized as set forth in figure 1.

Due to the importance of hepatic elimination of taxanes by the cytochrome P450 family, either an induction or inhibition of the cytochrome P450 isoenzymes involved in the metabolism could result in either an increase or decrease of the clearance of these drugs.

As such, the unfavorable pharmacokinetics of taxanes, due to rather rapid hepatic metabolism, results in the need for more frequent and/or higher than desirable doses for these drugs.

Therefore, inhibition of cytochrome P450 mediated taxanes metabolism will improve the pharmacokinetics (i.e. decrease clearance) of these drugs.

Several drugs have been described in the art as inhibitors of cytochrome P450 (iso)enzymes, and concomitant treatment of these drugs proved to alter the pharmacokinetics of drugs whose elimination is cytochrome P450 dependent.

See, as an example, the use of Ritonavir as cytochrome P450 inhibitor as reported in WO 97/01349 in the name of Abbott Laboratories.

In this respect, for therapeutic purposes, any inhibitor of cytochrome P450 mediated taxane metabolism, should be an inhibitor of both (CYP)2C8 and (CYP)3A4. At present, however, no such clinically-usable inhibitors have been reported as yet.

We unexpectedly found that the co-administration of estramustine phosphate (Estracyt®), an estradiol-17-[beta]-phosphate derivative widely used in the treatment of patients with advanced prostate cancer, resulted to be particularly effective in potentiating the therapeutic efficacy of taxanes. The said beneficial therapeutic effects, therefore, allow much lower and/or frequent doses of taxanes to be administered to a patient in need thereof.

Estramustine phosphate is a pro-dug that is converted to two main active metabolites: initially, estramustine phosphate is hydrolyzed to estramustine which, in turn, is metabolized by oxidation to estromustine.

As per the pro-drug itself, we found that also the main metabolites estramustine and estromustine resulted highly effective in inhibiting both (CYP)2C8 and (CYP)3A4 isoenzymes responsible for taxanes metabolism and clearance.

Therefore, it is a first object of the present invention the use of estramustine phosphate or metabolites thereof in the preparation of a medicament which potentiates the therapeutic efficacy of taxanes.

The said therapeutic effect, in particular, is exerted through (CYP)2C8 and (CYP)3A4 enzymes inhibition.

As above reported, the inhibitory activity towards (CYP)2C8 and (CYP)3A4 enzymes, hence leading to an improvement of the pharmacokinetic and pharmacodynamic profile of co-administered taxanes, has been observed with estramustine phosphate itself as well as with its metabolites estramustine and estromustine. Therefore, also the use of these latter in potentiating the therapeutic efficacy of taxanes is within the scope of the invention.

The dosage of estramustine phosphate or metabolites according to the invention, either as a single administration or repeated in a serial manner, will depend upon several factors such as, for instance, the selected schedule treatment comprising the therapy with taxanes.

High doses of estramustine phosphate or metabolites are however preferred.

Estramustine phosphate is a drug already known for both intravenous and oral administration. In this respect, the use of estramustine phosphate or metabolites thereof in potentiating the therapeutic efficacy of taxanes, according to the present invention, can also be accomplished by administering the drug through intravenous or oral route.

Of course, as oral estramustine phosphate is rapidly first-pass metabolized into estramustine and estromustine, it is clear to the man skilled in the art that the use of oral estramustine phosphate in inhibiting (CYP)2C8 and (CYP)3A4 enzymes, hence leading to the desired therapeutic effect, is only exerted by the estramustine phosphate metabolites estramustine and estromustine.

On the other side, when referring to an intravenous administration of estramustine phosphate, the above inhibitory activity appears to be exerted by the prodrug estramustine phosphate itself as well as by the two metabolites estramustine and estromustine.

According to a preferred embodiment of the invention, therefore, the use of estramustine phosphate and metabolites thereof is intended for intravenous administration.

In the present description and in any embodiment of the present invention, unless otherwise specified, when referring to estramustine phosphate or metabolites administered through intravenous route, we intend any intravenous infusion given as a bolus, otherwise solely referred to as i.v. push, or as a slow infusion given for a time varying from about 30 minutes to about 3 hours.

According to another preferred embodiment of the invention, any single intravenous infusion of estramustine phosphate or metabolites is intended at high doses, for instance exceeding 1300 mg or 950 mg/m^2.

Taxanes, whose therapeutic efficacy is potentiated according to the present invention, are those metabolized by cytochrome P450 enzymes such as, for instance, paclitaxel or docetaxel, independently from their administration route, formulation or schedule treatment comprising them.

As an example, the above taxanes can be formulated according to conventional means for intravenous administration or, alternatively, encapsulated within liposomes.

According to a preferred embodiment of the invention, the use of estramustine phosphate or metabolites thereof is intended to potentiate the therapeutic efficacy of paclitaxel.

According to another preferred embodiment of the invention, the use of estramustine phosphate or metabolites thereof is intended to potentiate the therapeutic efficacy of taxotere.

It is a further object of the present invention a formulation of estramustine phosphate or metabolites thereof for use in potentiating the therapeutic efficacy of taxanes by inhibiting (CYP)2C8 and (CYP)3A4 enzymes.

As set forth above, a preferred formulation of the invention comprises estramustine phosphate or metabolites for intravenous use.

The said formulations are used in therapy in the treatment of cancer such as, for instance, prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer and cancers of the brain.

A further object of the present invention is a combination which potentiates the therapeutic efficacy of taxanes by inhibiting (CYP)2C8 and (CYP)3A4 enzymes, which combination comprises estramustine phosphate or metabolites thereof for administration on the day of, or within 3 days of, administration of the taxane derivative.

Preferably, the said combination comprises the intravenous administration of estramustine phosphate or metabolites thereof.

Still another object of the invention is a product comprising estramustine phosphate or metabolites thereof and a taxane, as a combined preparation for simultaneous, separate or sequential use in anticancer therapy, wherein the said product is intended for potentiating the efficacy of the above taxane by improving its pharmacokinetic and pharmacodynamic profile.

Pharmaceutically acceptable carriers or excipients to be utilized in the preparation of a medicament or pharmaceutical composition according to the invention are well known to people skilled in the art of formulating compounds in a form of pharmaceutical compositions.

For example, such pharmaceutical compositions may routinely contain, e.g. pharmaceutically acceptable salts, buffering agents, preservatives and/or compatible carriers, especially those used in intravenous formulations. As used herein, "pharmaceutically acceptable carrier" refers to one or more compatible solid or liquid filler, diluent or encapsulating substances which are suitable for administration to mammals including humans.

Pharmaceutical compositions suitable for parenteral administration are typically formulated in a sterile form. The sterile composition thus may be a sterile solution or suspension in a non-toxic parenterally acceptable diluent or solvent.

Optionally, the aforementioned product, combination, formulation or use according to the present invention may further comprise another chemotherapeutic agent such as, for instance, CPT-11, SN-38, camptothecin derivatives, anthracycline glycosides, e.g., doxorubicin, idarubicin, epirubicin, etoposide, navelbine, vinblastine, carboplatin, cisplatin, celecoxib,. parecoxib, rofecoxib, valecoxib, JTE 5222, Sugen SU-5416, Sugen SU-6668, Herceptin, and the like, optionally within liposomal formulations thereof.

Brief description of figures

Figure 1: metabolic pathway of paclitaxel biotransformation in human;

Figure 2: inhibition of CYP2C8 mediated 6α-hydroxylation of paclitaxel by estromustine and estramustine at 100 μM. Results are presented as percentage of activity remaining in the presence of the compound. Values are mean ± SD (N=3).

Pharmacology

To study drug-drug interactions with taxanes it is not possible to use rodent models, either in vivo or in vitro, because of the metabolism of taxanes in rats and mice which is qualitatively and quantitatively different from that in humans (Monsarrat, 1993; Eiseman, 1994; Sparreboom, 1996).

Thus, for a taxane drug such as, for instance, paclitaxel, the need for carrying out in vitro studies using human derived material is clearly evident.

The key rationale for performing in vitro experiments on drug-drug interactions is the presumed applicability to the clinical situation. At present there is a growing body of literature that indicates that in many cases, in vitro studies are predictive of results in vivo (Hoener, 1994; Houston, 1994).

Although in vitro studies suggested that paclitaxel is mainly metabolized by CYP2C8 and to a lesser extent by CYP3A4 (Cresteil, 1994; Rahman, 1994) it has been recently shown that the role of CYP3A4 in vivo is as important as of CYP2C8 (Monsarrat, 1998).

The apparent discrepancy between the in vitro and in vivo observation might be due to induction of CYP3A4. Cancer patient receiving paclitaxel, in fact, are commonly pretreated with corticosteroids, known as inducers of CYP3A4 enzymes.

Whether in vitro inhibition of the two enzymes involved in taxanes metabolism, CYP3A4 and CYP2C8; is relevant for in vivo situation, highly depends on the plasma concentration levels of estramustine phosphate, estramustine and estromustine.

Nowadays, high estramustine phosphate doses are considered in advanced cancer therapy. For instance, doses of estramustine phosphate up to 2000 mg/m^2 result in plasma levels of both estramustine and estromustine well above 10 μM. As both estramustine phosphate and the two major metabolites, estramustine and estromustine appear to inhibit cytochrome P450, it should be realized the importance of the sum of the plasma levels of these three compounds.

As previously indicated, therefore, since both CYP3A4 and CYP2C8 enzymes play a primary role in the disposition of taxanes, inhibition of both CYP3A4 and CYP2C8 mediated enzyme activities by estramustine phosphate or metabolites thereof, preferably at high doses, would result in a decreased hepatic metabolism of taxanes and, as a consequence, a decreased excretion.

With the aim of better illustrating the present invention, without posing any limitation to it, herewith reported are some examples showing the inhibitory activity towards specific cytochrome P450 enzymes of estramustine phosphate, estramustine and estromustine.

Example 1

Chemicals

^{14}C-Chlorzoxazone, [phenylacetic acid ring-U-^{14}C]diclofenac sodium, S-[4-^{14}C] mephenytoin, [4-^{14}C]testosterone, were purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). and ^{14}C-Delavirdine mesylate (PNU-90152E) was obtained from Pharmacia & Upjohn, Kalamazoo, MI, USA. Human CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, were purchased from Gentest (Woburn, MA, USA). Estramustine phosphate, estramustine and estromustine were either commercially available or, alternatively, prepared according to well known methods.

Other reagents and solvents were analytical grade and were commercially available.

Incubation

The ability of estramustine phosphate, estramustine and estromustine to inhibit P450 enzymes was investigated in vitro against five different cDNA expressed human cytochrome P450 (CYP) enzyme systems (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4). CYP enzyme was used in an amount giving a turnover of the marker substrate of 10-20%, while the substrate concentration corresponded to its K_m value (see table 1).

Table 1

Incubation conditions for in vitro inhibition assay

Enzyme	Pmol/well	Substrate	µM	Incubation time (min)
CYP1A2	2	Chlorzoxazone	8.5	30
CYP2C9	0.35	Diclofenac	10	90
CYP2C19	3	(S)-mephenytoin	20	90
CYP2D6	0.7	Delavirdine	10	90
CYP3A4	0.65	Testosterone	50	90

Estramustine phosphate was dissolved in 0.1 M KH_2PO_4 (pH 7.4), whereas estromustine and estramustine were dissolved in DMSO:CH_3CN (1:1). The reaction, in a final volume of incubation of 100 µl, was started by adding NADPH. After 90 min incubation (30 min for CYP1A2) at 37 °C, the reaction was stopped by adding 50 µl CH_3CN followed by additional 50 µl of mobile phase. At the end, samples were centrifuged at 1200 *g* for 15 min at 4 °C and radio-HPLC analyzed. All incubations were conducted in triplicate.

Radio-HPLC analyses

Quantitation of substrates and their metabolites were achieved using an HPLC system equipped with a Radiomatic Flo-One radioactivity flow detector (Packard). Analytical separations of substrates and metabolites were performed on a Zorbax SB-C8 column, 4.6 x 150mm, 5µm (Hewlett Packard, Waldbronn, Germany), plus a 3Nucleosil 120-3 C18, 4 x 30mm precolumn (Macherey-Nagel, Duren, Germany). For testosterone,

analytical separations of substrate and metabolite were performed on a Zorbax SB-C18 column, 4.6 x 150mm, 5µm (Hewlett Packard), plus a 3Nucleosil 120-3 C18, 4 x 30mm precolumn (Macherey-Nagel). See table 2 for HPLC mobile phase conditions.

Table 2

Mobile phase and flow conditions for the radio-HPLC analyses

Assay	**Mobile phase A** [H_2O:CH_3OH:CH_3COOH =90:10:0.2] **Mobile phase B** [CH_3OH:H_2O:CH_3COOH =90:10:0.2]		**Flow** (ml/min)
	A (%)	**B (%)**	
Chlorzoxazone	40	60	1
(S)-mephenytoin	40	60	1
Delavirdine	30	70	1
Diclofenac			1.5
Time (min)			
0	80	20	
7	10	90	
10	10	90	
11	80	20	
13	80	20	
Assay	**Mobile phase A** [CH_3CN:H_2O:CH_3COOH =20:80:0.25] **mobile phase B** [CH_3CN:H_2O:CH_3COOH =65:35:0.25]		**Flow** (ml/min)
	A (%)	**B (%)**	
Testosterone	20	80	1

The resulting inhibitory effect exerted by estramustine phosphate, estramustine and estromustine against several human CYP mediated enzyme activities is presented in table 3.

Table 3

Inhibition of major human cytochrome P450 enzymes by estramustine phosphate, estromustine, and estramustine at either 10 or 100 µM. Results are presented as

percentage of activity remaining in the presence of the compound. Values are mean ± SD (n=3). Control (absence of the compound) values are 100%.

CYP	Estramustine phosphate		Estromustine		Estramustine	
	10 μM	100 μM	10 μM	100 μM	10 μM	100 μM
CYP1A2	99.8 ± 22.8	61.3 ± 11.9	85.0 ± 4.9	68.4 ± 8.0	72.7 ± 11.4	57.2 ±17.3
CYP2C9	88.6 ± 17.3	71.6 ± 10.2	92.5 ± 3.1	86.9 ± 7.2	56.4 ± 1.4	58.8 ± 3.8
CYP2C19	93.9 ± 2.6	87.5 ± 4.5	90.6 ± 2.1	81.0 ± 9.1	37.9 ± 10.0	40.1 ± 5.0
CYP2D6	87.1 ± 0.9	70.7 ± 7.0	64.1 ±19.9	76.9 ± 2.81	38.0 ± 5.6	32.7 ± 25.5
CYP3A4	80.7 ± 2.5	47.9 ± 4.8	43.2 ± 4.8	33.1 ± 1.6	27.0 ± 2.3	20.2 ± 4.3

From the above table clearly appears that at a concentration of 100 μM, all the compounds were able to inhibit CYP activities.

In particular, 6β-hydroxylation of testosterone (CYP3A4) was affected and an inhibition of approximately 80% was observed with estramustine. Of all the three compounds, estramustine resulted to be the most potent inhibitor.

Example 2

Chemicals

Paclitaxel was obtained from Sigma. Human CYP2C8, was purchased from Gentest (Woburn, MA, USA). Estramustine and estromustine Pharmacia & Upjohn (Nerviano, Italy). Other reagents and solvents were analytical grade and were commerical available.

Incubations

A 100 μl reaction mixture containing 4 pmol CYP2C8, 20 μM paclitaxel (5 mM paclitaxel stock in ethanol) and NADPH (2 mM) in 50 mM potassium phosphate buffer (pH 7.4) was incubated at 37°C for 40 min in the absence (solvent only) and presence of either 100 μM estramustine or estromustine.

Estramustine and estromustine were dissolved in DMSO:CH_3CN (1:1). The reaction was stopped by addition of 50 μl CH_3CN followed by an additional 50 μl of mobile phase. Finally samples were centrifuged at 1200 *g* for 15 min at 4°C and analyzed radio-HPLC. All incubations were conducted in triplicate.

HPLC analyses

Quantitation of substrates and their metabolites were achieved using an HPLC system equipped with a UV detector. 125 µl of the supernatent was injected into a Zorbax SB-C18 column, 4.6 x 150mm, 5µm (Hewlett Packard, Waldbronn, Germany), and separated at 45°C with a mobile phase initially of 58% methanol increasing to 82% methanol over 20 min and at a flow rate of 1.0 ml/min. See table 2 for HPLC mobile phase conditions. Both paclitaxel and the metabolite 6α-hydroxypaclitaxel were detected by its absorbance at 230 nm.

There is currently limited information regarding inhibitors, inducers and substrates of CYP2C8 in man, in vivo. Only a few clinically utilized drugs, for example paclitaxel and cerivastatin, are known to be substrates for CYP2C8 (Sonnichsen,1995; Wolfgang, 1998).

To investigate if estramustine and estromustine were able to inhibit CYP2C8 mediated enzyme activities, human CYP2C8 cDNA expressed microsomes were incubated with paclitaxel in the presence of either estramustine or estromustine.

In particular, at a concentration of 100 µM, estromustine or estramustine were able to inhibit 6α-hydroxylation of paclitaxel by 20% and 40%, respectively.

The results, expressed as percentage of activity remaining in the presence of the tested compound [values are mean ± SD (n=3)], are reported in figure 2.

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CLAIMS

1. Use of estramustine phosphate or a metabolite thereof in the preparation of a medicament which potentiates the therapeutic efficacy of a taxane.
2. Use according to claim 1 wherein the therapeutic efficacy of the taxane is potentiated by inhibiting (CYP)2C8 and (CYP)3A4 enzymes.
3. Use according to claim 1 wherein the medicament is formulated for the administration of estramustine phosphate or a metabolite thereof at a high dose.
4. Use according to claim 1 wherein the medicament is for intravenous administration.
5. Use according to claim 3 wherein the high dose of estramustine phosphate or a metabolite thereof is in excess of 1300 mg or 950 mg/m^2 for a single intravenous infusion.
6. Use according to claim 1 wherein the estramustine phosphate metabolite is estramustine or estromustine.
7. Use according to claim 1 wherein the taxane is selected from paclitaxel and docetaxel, both optionally encapsulated within liposomes.
8. Use according to claim 1 wherein the taxane is paclitaxel.
9. An agent for use in potentiating the therapeutic efficacy of a taxane comprising estramustine phosphate or a metabolite thereof.
10. An agent according to claim 9 wherein the therapeutic efficacy of the taxane is potentiated by inhibiting (CYP)2C8 and (CYP)3A4 enzymes.
11. An agent according to claim 9 comprising a high dose of estramustine phosphate or a metabolite thereof.
12. An agent according to claim 9 for intravenous use.
13. An agent according to claim 12 formulated to deliver a single intravenous infusion dosage of estramustine phosphate or a metabolite thereof in excess of 1300 mg or 950 mg/m^2.
14. An agent according to claim 9 wherein the estramustine phosphate metabolite is estramustine or estromustine.
15. An agent according to claim 9 wherein the taxane is selected from paclitaxel and docetaxel, both optionally encapsulated within liposomes.

16. An agent according to claim 9 wherein the taxane is paclitaxel.

17. An agent according to claim 9 for use in the treatment of cancer.

18. An agent according to claim 17 wherein the cancer is selected from prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer and cancers of the brain.

19. An agent according to claim 9 which is in the form of a pharmaceutical composition which further includes a pharmaceutically acceptable carrier or diluent.

20. A combination which potentiates the therapeutic efficacy of a taxane which comprises estramustine phosphate or a metabolite thereof for administration on the day of, or within 3 days of, administration of the said taxane.

21. A combination according to claim 20 wherein the therapeutic efficacy of the taxane is potentiated by inhibiting (CYP)2C8 and (CYP)3A4 enzymes.

22. A combination according to claim 20 wherein estramustine phosphate or the metabolite thereof is formulated for administration at a high dose.

23. A combination according to claim 20 wherein estramustine phosphate or the metabolite thereof is formulated for intravenous administration.

24. A combination according to claim 23 wherein the dosage of estramustine phosphate or a metabolite thereof is in excess of 1300 mg or 950 mg/m^2 for a single infusion.

25. A combination according to claim 20 wherein the estramustine phosphate metabolite is estramustine or estromustine.

26. A combination according to claim 20 wherein the taxane is selected from paclitaxel and docetaxel, both optionally encapsulated within liposomes.

27. A combination according to claim 20 wherein the taxane is paclitaxel.

28. A combination according to claim 20 for use in the treatment of cancer.

29. A combination according to claim 28 wherein the cancer is selected from prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer and cancers of the brain.

30. A product comprising estramustine phosphate or a metabolite thereof and a taxane as a combined preparation for simultaneous, separate or sequential use in anticancer therapy.

31. A product according to claim 30 wherein the said estramustine phosphate or metabolite thereof serves to potentiate the therapeutic efficacy of the said taxane.

32. A product according to claim 31 wherein the therapeutic efficacy of the taxane is potentiated by inhibiting (CYP)2C8 and (CYP)3A4 enzymes.

33. A product according to claim 30 comprising an additional chemotherapeutic agent.

34. A product according to claim 33 wherein the additional chemotherapeutic agent is selected from CPT-11, doxorubicin, etoposide, navelbine, vinblastine carboplatin and cisplatin.

35. A product according to claim 30 wherein the estramustine phosphate or metabolite thereof is formulated for intravenous use.

36. A product according to claim 35 wherein the dosage of estramustine phosphate or the metabolite thereof is in excess of 1300 mg or 950 mg/m^2 per single infusion.

37. A product according to claim 30 wherein the estramustine phosphate metabolite is estramustine or estromustine.

38. A product according to claim 30 wherein the taxane is selected from paclitaxel and docetaxel, both optionally encapsulated within liposomes.

39. A product according to claim 30 wherein the taxane is paclitaxel.

40. A method of treating a patient in need of taxane therapy, which method comprises administering estramustine phosphate or a metabolite thereof and a taxane to the said patient.

41. A method of potentiating the therapeutic efficacy of a taxane, which method comprises administering to a patient in need thereof a taxane and estramustine phosphate or a metabolite thereof.

42. A method according to claim 40 or 41 wherein the taxane and the estramustine phosphate or metabolite thereof are administered simultaneously, separately or sequentially to the said patient.

43. A method according to claim 40 or 41 wherein the estramustine phosphate or metabolite thereof and the taxane are administered as a combined preparation for simultaneous, separate or sequential use.

44. A method according to claim 40 or 41 wherein estramustine phosphate or the metabolite thereof is administered intravenously on the day of, or within 3 days of, administration of the taxane.

45. A method according to claim 40 or 41 wherein the estramustine phosphate metabolite is estramustine or estromustine.

46. A method according to claim 40 or 41 wherein the taxane is selected from paclitaxel and docetaxel, both optionally encapsulated within liposomes.

47. A method according to claim 40 or 41 wherein the taxane is paclitaxel.

48. Use of estramustine phosphate or a metabolite thereof in the preparation of a medicament which modifies the systemic exposure or the pharmacokinetic profile of a taxane.

Fig. 1

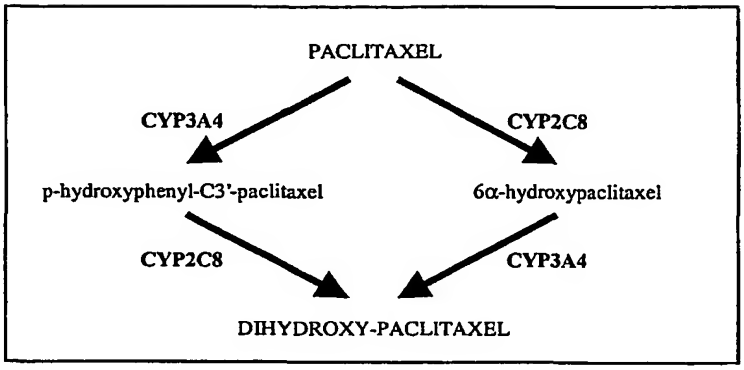
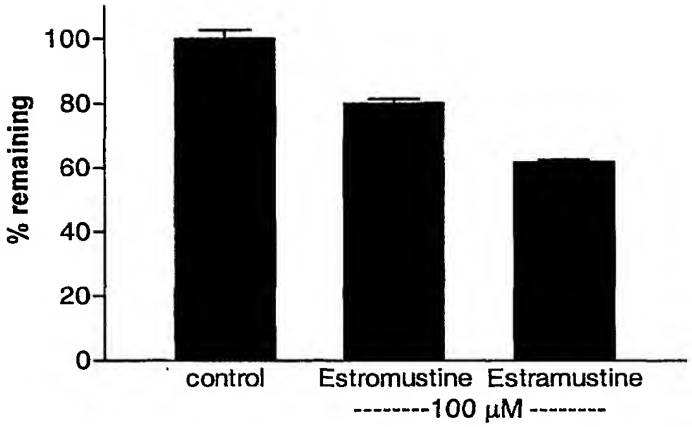

PACLITAXEL
CYP3A4
CYP2C8
p-hydroxyphenyl-C3'-paclitaxel
6α-hydroxypaclitaxel
CYP2C8
CYP3A4
DIHYDROXY-PACLITAXEL

Fig. 2


% remaining
100
80
60
40
20
0
control
Estromustine
Estramustine
--------100 µM --------

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/01088

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/66 A61K31/335 A61P35/00 //(A61K31/66,31:335)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, EMBASE, CHEM ABS Data, PHARMAPROJECTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 49869 A (ASP BERYL ;FREDHOLM BO (SE); GUNNARSSON PER OLV (SE); UPJOHN CO (U) 7 October 1999 (1999-10-07) *cf. abstract, page 7, lines 1-14, page 13, lines 8-16, 23/24, claims 1,9,14,62-64*	1-48
X	WO 97 44026 A (NEUROMEDICA INC) 27 November 1997 (1997-11-27) *cf. abstract, page 4 bridging with page 5, lines 1-20, page 18, lines 6-24, page 19, line 3, page 20, line 7*	1-48

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2001	26/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Stoltner, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

International Application No
PCT/EP 01/01088

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